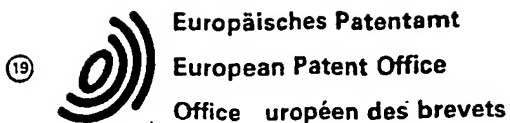


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(54) Injectable composition of rapamycin.

(57) Described is an injectable composition comprising rapamycin and a nonionic surfactant selected from polyoxyethylated fatty acids, polyoxyethylated fatty alcohols or polyoxyethylated glycerine hydroxy fatty acid esters and a method of preparing said compositions. The composition provides enhanced blood levels of rapamycin in a mammal following injected administration thereof.

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This invention relates to a novel injectable composition of rapamycin which provides a high order of rapamycin in the blood of a mammal following injected administration of the composition. More specifically, 5 the herein disclosed invention relates to new compositions of rapamycin containing a non-ionic surfactant. The invention further relates to a process for preparing and a method for using the disclosed composition.

Rapamycin is an antifungal antibiotic described by 10 C.Vezina et al., J. Antibiot., 28, 721(1975), S.N. Sehgal et al., J. Antibiot., 28, 727 (1975), S.N. Sehgal et al., U.S. Patent 3,929,992, issued December 30, 1975 and S.N. Sehgal et al., U.S. Patent 3,993,749, issued November 23, 1976. The latter two patents are herein incorporated 15 by reference. Rapamycin is extracted from a streptomycete (Streptomyces hygroscopicus NRRL 5491) isolated from an Easter Island soil sample and is particularly effective against Candida albicans both in vitro and in vivo, H.A.Baker et al., J. Antibiot., 31, 539 (1978). 20 Streptomyces hygroscopicus NRRL 5491 samples were deposited 23rd June 1972 without restrictions with the Northern Utilization and Research Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois, U.S.A. A report by R.R. Martel et al., Can.J. Physiol., 25 55, 48 (1977) describes the use of rapamycin for the prevention of the development of experimental immunopathies. Recently rapamycin was shown to be an effective agent for treating carcinogenic tumors in a mammal by S.N. Sehgal and C.Vezina, United States Patent Application 30 Serial No. 957,626, filed November 3, 1978, herein incorporated by reference. In Belgium, a corresponding application of the latter application issued as Belgium Patent No. 877700 on January 14, 1980.

Rapamycin and compositions thereof, as described in the above reports, are virtually insoluble in aqueous solutions and are not suitable for injection. The

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composition of this invention provides rapamycin in a water soluble formulation which is suitable for injection, especially intravenous.

A number of drugs having poor water solubility have been formulated with nonionic surfactants. Polyoxyethylated glycerin hydroxy fatty acids, such as sold under the trade mark Cremophor, are examples of such nonionic surfactants. Cremophor was shown to be an effective solvent for the prevention of i.v. diazepam-induced thrombophelbitis by M.A.K. Mattila et al., Br.J. Anaesth., 51,891(1979). Cremophor was used as a solubilizing agent for an injectable anaesthetic by B. Kay and G. Rolly, Acta. Anaesth. Belg., 28,303 (1977) and B. Kay and G. Rolly, Acta. Anaesth. Belg., 28,317(1977). The antifungal agent, Monistat i.v., is formulated with PEG 40 castor oil, "Physicians' Desk Reference", Publisher Charles E. Baker, Jr., Published by Medical Economics Company, Oradel, New Jersey, 33 ed., 1979, p. 1255. Formulations of ergot alkaloids with a large number of nonionic emulsifiers are described by J. Franz, United Kingdom Patent 1,513,383, published June 7, 1978. Suggested uses and technical description of the Cremophor surfactants are described in the following Technical Leaflets: "Cremophor EL", M 1714 e, (7063) May 1977 (RB), 5th Revision, BASF Aktiengesellschaft, D-6700 Ludwigshafen, Federal Republic of Germany and "Cremophor RH Grades", M 5629 e, April 1978 (JWF), BASF Aktiengesellschaft, D-6700 Ludwigshafen, Federal Republic of Germany. Rapamycin, per se, does not readily go into solution in polyoxyethylated glycerin hydroxy fatty acids. The present invention relates to a new injectable composition of rapamycin which is neither disclosed in, nor rendered obvious by, any of the above cited reports, or other references of which we are aware.

According to the present invention, an injectable composition of rapamycin is provided comprising a therapeutically effective amount of rapamycin and a

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nonionic surfactant selected from polyoxyethylated fatty acids, polyoxyethylated fatty alcohols or polyoxyethylated glycerine hydroxy fatty acid esters. A preferred injectable composition comprises a therapeutically effective amount of rapamycin in an aqueous solution of 1.0 to 50 per cent by weight of the nonionic surfactant. A more preferred injectable composition contains an aqueous solution of 1.0 to 35 per cent by weight of the nonionic surfactant. A most preferred composition contains an aqueous solution of 1.0 to 20 per cent by weight of the nonionic surfactant. The above compositions can also contain pharmaceutically acceptable excipients commonly used in pharmaceutical formulations.

The preferred method of injection is by intravenous injection.

The injectable rapamycin composition of this invention provides a therapeutically effective amount of rapamycin in the blood of a mammal and also provides a therapeutically effective amount of rapamycin in the brain, liver, kidney, lung and spleen of a mammal.

The invention also provides a method of making the rapamycin-containing nonionic surfactant injectable compositions. These injectable compositions of rapamycin are prepared by (a) dissolving rapamycin in an organic solvent, which is capable of dissolving rapamycin and is miscible with the nonionic surfactant, (b) adding the nonionic surfactant, (c) if required, removing the organic solvent, and (d) adding water or an aqueous solution containing pharmaceutically acceptable excipients commonly used in pharmaceutical formulations.

The injectable rapamycin composition comprises the following three main components: rapamycin, a nonionic surfactant and water.

The therapeutically effective amount of rapamycin in the injectable composition is usually in the range of

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about 1.0 to about 1.0 to about 20 mg/ml of solution. This range represents a range of about 0.1 to about to about 2.0 per cent by weight of rapamycin in the injectable composition.

5       The nonionic surfactant can be in the range of about 1.0 to about 50 per cent by weight of the injectable composition. In good pharmaceutical practice, the amount of solubilizing agents and excipients should be kept at a minimum, thus the  
10       amount of the nonionic surfactant in the composition should be dependent on the desired amount of rapamycin in the composition.

Nonionic surfactants used in the injectable composition of rapamycin increase the solubility of  
15       rapamycin in aqueous solutions. Two or more nonionic surfactants can also be used in the injectable composition of rapamycin. Nonionic surfactants for use in the composition are selected from polyoxyethylated fatty acids; polyoxyethylated fatty alcohols; and  
20       polyoxyethylated glycerin hydroxy fatty acid esters, e.g. polyoxyethylated castor oil, exemplified by Cremophor EL and polyoxyethylated hydrogenated castor oil, exemplified by Cremophor RH and Cremophor RH 60. Polyoxyethylated castor oil is prepared by reacting ethylene oxide with  
25       castor oil, for example, Cremophor EL is the product obtained from the reaction of 35 moles of ethylene oxide with one mole of castor oil. Similarly, polyoxyethylated hydrogenated castor oil is obtained by reacting ethylene oxide with hydrogenated castor oil, for example  
30       Cremophor RH 60 is prepared by reacting 60 moles of ethylene oxide with one mole of hydrogenated castor oil. In a similar manner, other polyoxyethylated fatty acids or fatty alcohols can be prepared by the reaction of ethylene oxide with a fatty acid, for example, stearic acid or with a fatty alcohol, for example, cetyl

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alcohol. Preferred nonionic surfactants in the composition are selected from polyoxyethylated castor oil and polyoxyethylated hydrogenated castor oil.

The rapamycin composition of the invention is prepared by the discovery of a method to dissolve rapamycin in a nonionic surfactant so that when diluted with water, rapamycin remains in solution. Rapamycin is insoluble in most surfactants, including the nonionic surfactants. Rapamycin is first dissolved in an organic solvent miscible with the surfactant; preferably the solvent is one that can be removed easily by distillation. Examples of these solvents are acetone, methanol, ethanol and the like. Thereafter the rapamycin containing solvent and the surfactant are admixed. If desired or if the solvent is not pharmaceutically acceptable, the solvent is removed from the solution containing rapamycin, nonionic surfactant and solvent. A preferred method of removing the solvent is by evaporation wherein the solution is subjected to reduced pressure at 20 to 50°C until the solution remains at constant weight. Upon removal of the solvent, the rapamycin remains in solution in the nonionic surfactant. Dilution of the above solutions containing rapamycin, solvent and nonionic surfactant or rapamycin and nonionic surfactant with water or an aqueous solution containing pharmaceutically acceptable excipients provides compositions suitable for injection. This dilution is preferably made shortly before administration, e.g. within four hours before injection. Another mode of preparing the injectable solution would be to admix a solution of the surfactant in water with the rapamycin-containing organic solvent.

Accordingly this invention provides a process for preparing an injectable rapamycin composition as hereinbefore described which comprises dissolving rapamycin in an organic solvent which is capable of dissolving rapamycin

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and is miscible with the nonionic surfactant

and either (i) adding the nonionic surfactant,  
if required removing some or all of the  
organic solvent, and adding water;

5 or (ii) adding a solution of nonionic surfactant  
in water;

the amounts of rapamycin and nonionic surfactant being  
predetermined to give the desired concentrations in the  
composition.

10 Pharmaceutically acceptable excipients as used  
herein are the additives which provide isotonic composition,  
for example, saline, Ringer's solution, glucose and the  
like; preservatives; and the like.

The rapamycin composition can also contain other  
15 nonionic surfactants, for example, polysorbate 80,  
propylene glycol and/or polyethylene glycol.

The following examples illustrate further this  
invention where percentages relating to final compositions  
are on a w/w basis.

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EXAMPLE 1

PREPARATION OF INJECTABLE RAPAMYCIN COMPOSITIONS

A. Rapamycin Compositions Without Organic Solvent

5        Rapamycin (100 mg) was dissolved in ethanol (4 ml) and to  
this solution, polyoxyethylated castor oil (Cremophor EL, 2ml  $\approx$  2g,  
density = 1.05 g/ml @ 25°C) was added. The  
solution was subjected to evaporation under reduced pressure at 50°C until  
the ethanol was removed to give a solution containing 50 mg of rapamycin  
10 per millilitre of Cremophor EL. To 2 ml of the latter solution, water (8  
ml) was added to provide a solution containing 10 mg of rapamycin per millilitre  
of aqueous 20% Cremophor EL.

In the same manner, but replacing ethanol with an equivalent  
amount of acetone or methanol, the latter solution of rapamycin was obtained.

15        Similarly, by replacing water with physiological saline, a solution  
containing 10 mg of rapamycin and 20% Cremophor EL in physiological saline  
is obtained.

Similarly, by using 10, 20, 50, 150 or 200 mg of rapamycin, the  
following solutions are obtained, respectively: 1 mg of rapamycin per millilitre  
20 of aqueous 20% Cremophor EL, 2 mg of rapamycin per millilitre of aqueous  
20% Cremophor EL, 5 mg of rapamycin per millilitre of aqueous 20% Cremophor  
EL, 15 mg of rapamycin per millilitre of aqueous 20% Cremophor EL,  
and 20 mg of rapamycin per millilitre of aqueous 20% Cremophor EL.

Similarly, by replacing polyoxyethylated castor oil with an equiv-  
25 alent volume of polyoxyethylated hydrogenated castor oil (Cremophor RH  
40 and Cremophor RH 60) or a mixture containing equal volumes of poly-  
oxyethylated castor oil (Cremophor EL) and polysorbate 80, the following  
injectable compositions are obtained, respectively: 10 mg of rapamycin per  
millilitre of aqueous 20% Cremophor RH 40, 10 mg of rapamycin per millilitre  
30 of aqueous 20% Cremophor RH 60, and 10 mg of rapamycin per millilitre of  
aqueous 10% Cremophor EL and 10% polysorbate 80.

Similarly, by using 20 mg of rapamycin and 1 ml of Cremophor  
EL or 50 mg of rapamycin and 2 ml of Cremophor EL, the following injectable  
compositions are obtained, respectively: 1 mg of rapamycin per millilitre of



aqueous 5% Cremophor EL and 5 mg of rapamycin per millilitre of aqueous 10% Cremophor EL.

5 B. Rapamycin Compositions With Organic Solvent

Rapamycin (1.0 g) was dissolved in a solution of ethanol (8.0 g), propylene glycol (32.0 g) and Cremophor RH (10.0 g). To this solution, was added 50.0 g of water. The resulting solution (100 ml) provided a composition containing 10 mg of rapamycin per millilitre of a solution composed of 8% ethanol, 32% propylene glycol, 10% Cremophor RH and 50% water.

10 Similarly, by using 1.5 g of rapamycin, the following injectable composition was obtained, 15 mg of rapamycin per millilitre of a solution composed of 8% ethanol, 32% propylene glycol, 10% Cremophor RH and 50% water.

15 C. Concentrated Rapamycin Solution With a Surfactant Diluent

Rapamycin (67.98 g) and butylated hydroxyanisole (1.24 g), as an antioxidant preservative, were dissolved in 930.78 g of nitrogen purged absolute ethanol. The solution was filtered through a bacterial filter and 1.1 ml of filtrate was filled into a 5 ml-amber glass ampule using a nitrogen flush. The ampule was sealed to protect the concentrated rapamycin solution from light and air.

20 Benzyl alcohol N.F. (33.34 g), as an antiseptic, and Cremophor EL (111.12 g) were added to sufficient water (USP) with mixing to make 1000 ml of a surfactant diluent solution. The diluent solution was filtered and 9 ml of the filtrate was filled into a 20 ml glass vial. Within four hours of injection, 1.0 ml of the contents of the ampule of concentrated rapamycin solution is withdrawn, via

25 a syringe, added to a vial of diluent solution, and shaken to form an injectable solution containing 5.5 mg rapamycin per ml.

EXAMPLE 2

ASSAY METHOD FOR DETERMINATION OF RAPAMYCIN IN THE SERUM AND TISSUE OF AN ANIMAL

30 A. Serum Concentrations

Medium: Nystatin assay agar (Antibiotic Medium 12), BBL 10,982. The test organism, Candida Albicans House AY F-598, was maintained by weekly transfer on Sabouraud's agar slopes. Slope cultures were incubated for 48 hours at 30° C and stored at 4° C until used. Test inoculum was prepared by subculturing the Candida strain in Sabouraud's broth and incubating overnight at 37° C.

35

Preparation of plates: In the assay, a modification of the anti-biotic assay method described by J.V. Bennett et al., Applied Microbiology, 14 (2), 170 (1966) was used. 200 Millilitre aliquots of molten Nystatin assay agar kept at 56° C were inoculated with 0.25 ml of a  $10^{-1}$  dilution of an overnight broth culture of C. albicans F-598. When the seeded agar solidified, 64 wells of 4.7 mm in diameter were punched at spaced intervals on an agar surface measuring 12" x 12"; this size of wells gave suitable zone diameters with the range of standards used. After the wells were filled with standards and samples, plates were covered, sealed and incubated for 18 hours at 37° C.

Standards: Rapamycin powder was weighed out in convenient amounts, and a stock solution prepared in methanol to give a concentration of 1,000 µg/ml; further dilutions were carried out in appropriate serum. Final concentration of standards in serum were: 5.0, 2.5, 1.25, 0.62 and 0.31 µg/ml respectively. Averages of a minimum of four separate zone diameter readings per standard concentration were plotted on semilog 3-cycle graph paper against the antibiotic concentrations. These five points served to draw the best fitting straight line. The concentrations of the unknown samples were calculated by obtaining the average diameters of at least four inhibition zones per sample and converting them to concentration by interpolation from the standard curve.

Sample Preparation: Similar to procedure for serum concentration.

Standards: A stock solution of rapamycin containing 1000 µg/ml in methanol was prepared. Further dilutions were carried out in methanol to give concentrations of 500, 250, 125, 62.5, 31.0 and 15.5 µg/ml, respectively. 0.1 Millilitre of the above concentrations was added to 1 gram sample of the various tissues: lung, liver, kidney, spleen and brain. To this mixture, 3.9 ml of saline was added to give a final concentration of 10, 5, 2.5, 1.25, 0.62 and 0.31 µg/gram of tissue. All samples were mixed by means of a Virtis No. 45 homogenizer, Virtis Research Equipment, Gardiner, New York. Averages of a minimum of four separate zone diameter readings per standard concentration were plotted on semi-log 3-cycle graph paper against the rapamycin concentrations. These five points served to draw the best fitting straight line. The con-

centrations of the unknown samples were calculated by obtaining the average diameters of at least four inhibition zones per sample and converting them  
5 to concentrations by interpolation from the standard curve.

C. Sample Collection

All formulations were prepared in the solvent and diluted with sterile distilled water prior to administration. Mice were sacrificed and bled by cardiac puncture and the following organs removed for tissue studies: brain,  
10 lung, liver, kidney and spleen. Standard curves for blood and tissue concentrations were prepared in the appropriate serum and tissue. Control mice (10) were administered each rapamycin formulation and were observed for a period of 60 days for deaths.

Sample Preparation: A one-gram sample was placed in a 30-ml  
15 Virtis Homogenizer cup and to this was added 4 ml of a 2% methanol in saline solution and the tissue was ground by the Virtis No. 45 Homogenizer to aid in sampling and to break down some of the connective tissue. This solution was used in the microbiological assay described above.

EXAMPLE 3

20 ADMINISTRATION OF THE RAPAMYCIN INJECTABLE COMPOSITIONS AND RESULTS

A. Test Animals and Mode of Administration

Mice, Swiss strain CD-1 (CBL, Montreal), 30 grams, male.

Intraperitoneal, 0.5 ml, peritoneal cavity.

25 Intravenous, 0.2 ml, tail vein.

Dog, beagle, 7.0 kg, female.

Intravenous administration, 5 ml, slow infusion, tibial vein.

Rat, Sprague Dawley (CBL, Montreal), 80 grams, male.

Intravenous administration, 0.5 ml, caudal vein.

30 B. Rapamycin in 8% ethanol plus 32% propylene glycol plus 10% Cremophor RH 40 plus 50% water

Concentration of rapamycin: 10 mg and 15 mg/ml.

Test animal: Mice, 6 per sample time for serum and tissue concentrations.

Dog, one sample per time interval.

5 Doses and administration: Mice 66.5 and 99.7 mg/kg single intravenous injection.

Dog, 7.1 mg/kg, single, slow intravenous injection.

Sample times: Mice 15, 30, 60 minutes, 1, 2, 4, 6, 7 and 8 hours.

Dog, 15, 30, 60 minutes, 2 and 4 hours

10 Assays: Mice, serum and tissue concentrations at sample times listed above.

Dog, serum concentrations at sample times listed above.

Results: Results of serum concentrations for doses of 66.5 and 99.7 mg/kg in mice are reported in Tables 1 and 2. Tissue concentrations in 15 mice are reported in Tables 1 and 2. Serum concentrations in a dog are reported in Table 3.

C. Rapamycin in 20% Cremophor EL plus 80% water

Concentration of rapamycin: 10 mg/ml.

20 Test animal: Mice, 6 per sample time for serum and tissue concentrations.

Doses and administration: Mice 33.3 and 66.5 mg/kg single intravenous injection.

Sample times: 15, 30 and 60 minutes, 2, 4, 6, and 24 hours.

25 Assays: Serum and tissue concentrations at sample times listed above.

Results: The results of the serum concentrations and tissue concentrations obtained with the doses of 33.3 and 66.5 mg/kg in mice are reported in Tables 4 and 5 respectively.

30 D. Rapamycin in 8% ethanol plus 32% propylene glycol plus 10% Cremophor RH 40 plus 50% water

Concentration of rapamycin: 10 mg/ml.

Test animal: Mice, 6 per sample time for serum and tissue concentrations.

35 Dose and administration: 160 mg/kg, single intraperitoneal injection.

Sample times: 30, 60 minutes, 2, 4, 6 and 24 hours.

Assays: Serum and tissue concentrations at sample times listed

above.

5

Results: Results of the serum concentration and tissue concentrations are reported in Table 6.

E. Rapamycin in 20% Cremophor EL plus 80% water

Concentration of rapamycin: 10 mg/ml.

Test animal: Rat, 2 per sample time.

10

Doses and administration: Rat, 62.5 mg/kg, single intravenous

injection.

Sample times: 15, 30 and 60 minutes, 2, 4 and 6 hours.

Assays: Tissue concentrations at sample times listed above.

Results: Results of the tissue concentrations are reported in

15 Table 7.

F. Rapamycin in 20% Cremophor EL plus 80% water

Concentration of rapamycin: 7.5 mg/ml.

Test animal: Rat, 2 per sample time.

20 injection.

Doses and administration: Rat, 46.8 mg/kg, single intravenous

Sample times: 15, 30 and 60 minutes, 2, 4 and 6 hours.

Assays: Serum and tissue concentrations at sample times listed

above.

25 Results: Results of the serum concentrations and tissue concentrations are reported in Table 8.

G. Rapamycin in 20% Cremophor EL plus 80% water

Concentration of rapamycin: 5.0 mg/ml.

Test animal: Rat, 2 per sample time.

30 injection.

Doses and administration: Rat, 31.25 mg/kg, single intravenous

Sample times: 15, 30 and 60 minutes, 2, 4 and 6 hours.

Assays: Serum and tissue concentrations at sample times listed

above.

35 Results: Results of the serum concentrations and tissue concentrations are reported in Table 9.

Table 1. Serum and Tissue Concentration after Intravenous Administration of Rapamycin in 8% Ethanol, 32% Propylene glycol, 10% Cremophor RH 40 and 50% Water in Mice at a Dose of 66.5 mg/kg.

5	TIME	SERUM <sup>a</sup>	BRAIN <sup>b</sup>	LIVER <sup>b</sup>	KIDNEY <sup>b</sup>	LUNG <sup>b</sup>	SPLEEN <sup>b</sup>
	15 min	19.5	1.32	8.8	17.0	5.7	2.0
	30 "	13.5	1.05	7.0	8.4	4.3	1.6
	60 "	7.9	0.49	5.1	5.6	2.7	0.86
10	2 hr	3.6	0.41	1.8	2.0	1.5	0.21
	4 "	2.35	0	0.46	0.97	0.76	0.1
	6 "	2.0	0	0.43	0.55	0.66	0.1
	7 "	2.77	0	trace	0.32	0.72	trace
	8 "	1.3	0	0	0	0.4	0
15	24 "	-	-	-	-	-	-
<sup>a</sup> Rapamycin in Serum, µg/ml							
<sup>b</sup> Rapamycin in Tissues, µg/g							

Table 2. Serum and Tissue Concentrations after Intravenous Administration of Rapamycin in 8% Ethanol, 32% Propylene glycol, 10% Cremophor RH 40 and 50% Water in Mice at a Dose of 99.7 mg/kg

20	TIME	SERUM <sup>a</sup>	BRAIN <sup>b</sup>	LIVER <sup>b</sup>	KIDNEY <sup>b</sup>	LUNG <sup>b</sup>	SPLEEN <sup>b</sup>
	15 min	29.2	1.7	14.2	11.2	7.0	10.0
25	30 "	26.5	1.7	8.0	11.0	5.2	6.7
	60 "	12.5	1.25	6.4	7.5	5.6	2.5
	2 hr	6.0	1.02	4.5	4.5	4.6	2.25
	4 "	3.4	0.49	2.85	2.95	5.0	0.8
	6 "	2.5	trace	0.62	0.53	7.4	0.1
30	7 "	-	-	-	-	-	-
	8 "	-	-	-	-	-	-
	24 "	-	-	-	-	-	-
<sup>a</sup> Rapamycin in Serum, µg/ml							
<sup>b</sup> Rapamycin in Tissues, µg/g							

Table 3. Serum Concentration after Intravenous Administration of Rapamycin in 8% Ethanol, 32% Propylene glycol, 10% Cremophor RH 40 and 50% Water in a Dog at a dose of 7.1 mg/kg

5

TIME	SERUM <sup>a</sup>
0	0
15 min	2.3
30 min	2.3
1 hr	0.95
2 hr	0.34
4 hr	0.135

10

<sup>a</sup> Rapamycin in Serum, µg/ml

15

Table 4. Serum and Tissue Concentrations after Intravenous Administration of Rapamycin in 20% Cremophor EL and 80% Water to Mice at a Dose of 33.3 mg/kg

TIME	SERUM <sup>a</sup>	BRAIN <sup>b</sup>	LIVER <sup>b</sup>	KIDNEY <sup>b</sup>	LUNG <sup>b</sup>	SPLEEN <sup>b</sup>
15 min	6.4	0	9.0	9.2	7.5	2.1
30 "	6.45	0	11.0	3.75	8.0	1.35
60 "	3.45	0	5.2	1.9	4.0	0.62
2 hr	3.47	0	2.0	0.44	1.2	0.26
4 "	1.65	0	0.86	0.16	0.56	0.1
6 "	2.2	0	0.4	Trace	0.28	0
24 "	0	0	0	0	0	0

<sup>a</sup> Rapamycin in Serum, µg/ml

<sup>b</sup> Rapamycin in Tissues, µg/g

30

Table 5. Serum and Tissue Concentrations after Intravenous Administration of Rapamycin in 20% Cremophor EL and 80% Water to Mice at a Dose of 66.5 mg/kg.

5

TIME	SERUM <sup>a</sup>	BRAIN <sup>b</sup>	LIVER <sup>b</sup>	KIDNEY <sup>b</sup>	LUNG <sup>b</sup>	SPLEEN <sup>b</sup>
15 min	18.0	0.68	16.0	10.0	19.0	8.3
30 "	12.35	0.83	8.5	6.1	14.5	7.5
10 60 "	11.75	0.4	12.5	7.0	10.0	3.75
2 hr	6.6	0.5	5.2	2.85	12.0	2.5
4 "	5.0	0	4.2	1.15	1.45	0.56
6 "	2.77	0	0.27	0.38	0.58	0.155
24 "	0	0	0	0	0	0

15 <sup>a</sup> Rapamycin in Serum, µg/ml

<sup>b</sup> Rapamycin in Tissues, µg/g

Table 6. Serum and Tissue Concentrations after Intraperitoneal Administration of Rapamycin in 8% Ethanol, 32% Propylene glycol, 10% Cremophor RH 40 and Water in Mice at a Dose of 160 mg/kg.

20

TIME	SERUM <sup>a</sup>	BRAIN <sup>b</sup>	LIVER <sup>b</sup>	KIDNEY <sup>b</sup>	LUNG <sup>b</sup>	SPLEEN <sup>b</sup>
15 min	-	-	-	-	-	-
30 "	8.85	0	6.5	7.2	8.0	6.4
25 60 "	17.0	0	10.0	4.3	15.3	6.4
2 hr	16.75	0	10.0	11.0	11.2	5.1
4 "	10.65	0	6.2	12.2	7.1	5.1
6 "	7.15	-	1.8	6.7	3.4	5.5
7 "	-	-	-	-	-	-
30 8 "	-	-	-	-	-	-
24 "	0.68	0	0	0	0.4	0.62

<sup>a</sup> Rapamycin in Serum, µg/ml

<sup>b</sup> Rapamycin in Tissues, µg/g

35



Table 7. Tissue Concentrations after Intravenous Administration of Rapamycin in 20% Cremophor EL and 80% Water to Rats at a Dose of 62.5 mg/kg

TIME	RAPAMYCIN (µg/g)				
	BRAIN	LIVER	KIDNEYS	LUNGS	SPLEEN
0	0	0	0	0	0
15 min	1.95	48.0	19.0	16.5	15.0
10 30 "	0.76	40.5	19.0	16.0	12.0
60 "	not available	16.0	6.7	9.2	4.05
2 hrs	not available	13.2	7.2	8.4	4.45
4 "	0.76	8.8	3.8	4.05	3.2
15 6 "	<0.6	6.0	3.2	3.0	1.8

Table 8. Serum and Tissue Concentrations after Intravenous Administration of Rapamycin in 20% Cremophor EL and 80% Water to Rats at a Dose of 46.8 mg/kg

TIME	SERUM <sup>a</sup>	BRAIN <sup>b</sup>	LIVER <sup>b</sup>	KIDNEY <sup>b</sup>	LUNG <sup>b</sup>	SPLEEN <sup>b</sup>
15 min	10.0 <sup>c</sup> -10.0 <sup>c</sup>	0	43.0	13.8	13.0	8.3
30 "	10.5-10.5	0	31.0	12.0	14.0	8.3
60 "	7.6-3.1	0.83	12.0	4.95	7.2	4.4
25 2 hr	6.0-2.7	0	5.2	2.15	3.0	1.6
4 "	2.2-1.25	0	5.2	2.15	3.0	1.6
6 "	0.45-1.0	0	5.2	2.15	3.0	1.6

<sup>a</sup> Rapamycin in Serum, µg/ml

<sup>b</sup> Rapamycin in Tissues, µg/g.

<sup>30c</sup> Values from individual rats

Table 9 Serum and Tissue Concentrations after Intravenous Administration of Rapamycin in 20% Cremophor EL and 80% Water to Rats at a Dose of 31.25 mg/kg

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TIME	SERUM <sup>a</sup>	BRAIN <sup>b</sup>	LIVER <sup>b</sup>	KIDNEY <sup>b</sup>	LUNG <sup>b</sup>	SPLEEN <sup>b</sup>
15 min	2.4 <sup>c</sup> -6.3 <sup>c</sup>	0	5.2	8.6	5.2	3.3
30 "	6.4-3.5	0	12.5	6.7	6.4	3.8
10 60 "	N.A.-3.6	0	8.7	5.0	6.3	2.3
2 hr	2.0-3.35	0	5.6	3.5	4.4	2.0
4 "	0.84-0.19	0	1.4	1.4	1.03	0.72
6 "	0.17-0.19	0	1.1	0.76	0.7	0.475

- <sup>a</sup> Rapamycin in Serum, µg/ml  
15 <sup>b</sup> Rapamycin in Tissues, µg/ml  
<sup>c</sup> Values from individual rats  
N.A. = not available

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CLAIMS

1. An injectable composition of rapamycin, suitable for intravenous administration, which comprises about 1 to 20 milligrams of rapamycin per millilitre of the composition and a nonionic surfactant selected from polyoxyethylated fatty acids, polyoxyethylated fatty alcohols and polyoxyethylated glycerine hydroxy fatty acid esters.
2. A composition of Claim 1 containing an aqueous solution of about 1 to 50 percent by weight of the nonionic surfactant.
3. A composition of Claim 1 containing an aqueous solution of about 1 to 20 percent by weight of the nonionic surfactant.
4. A composition as claimed in Claim 1 or Claim 2 wherein the nonionic surfactant is polyoxyethylated castor oil or polyoxyethylated hydrogenated castor oil.
5. A composition of Claim 1 containing about 1 to 20 milligrams of rapamycin per millilitre of an aqueous solution of about 1 to 20 percent by weight of a nonionic surfactant selected from polyoxyethylated castor oil and polyoxyethylated hydrogenated castor oil.
6. A process for preparing an injectable composition, suitable for intravenous administration, comprising about 1 to 20 milligrams of rapamycin per millimeter of an aqueous solution of a nonionic surfactant selected from polyoxyethylated fatty acids, polyoxy-

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ethylated fatty alcohols and polyoxyethylated glycerin hydroxy fatty acid esters, which comprises dissolving rapamycin in an organic solvent which is capable of dissolving rapamycin and is miscible with the nonionic surfactant, and either

- (i) adding the nonionic surfactant, if required removing some or all of the organic solvent, and adding water;

or

- (ii) adding a solution of nonionic surfactant in water;

the amounts of rapamycin and nonionic surfactant being predetermined to give the desired concentrations in the composition.

7. A process as claimed in Claim 6 wherein the organic solvent is acetone, methanol or ethanol.
8. A process as claimed in Claim 6 or Claim 7 wherein the nonionic surfactant is polyoxyethylated castor oil or polyoxyethylated hydrogenated castor oil.
9. A process as claimed in any one of Claims 6, 7 and 8 wherein the aqueous solution comprises about 1.0 to 20 percent by weight of nonionic surfactant.
10. An intravenously injectable composition as claimed in any one of Claims 1 to 5 for use as an agent for enhancing blood levels of rapamycin or for providing rapamycin in the brain of a mammal.

CLAIMS FOR AUSTRIA

1. A process for preparing an injectable composition, suitable for intravenous administration, comprising about 1 to 20 milligrams of rapamycin per millimeter of an aqueous solution of a nonionic surfactant selected from polyoxyethylated fatty acids, polyoxyethylated fatty alcohols and polyoxyethylated glycerin hydroxy fatty acid esters, which comprises dissolving rapamycin in an organic solvent which is capable of dissolving rapamycin and is miscible with the nonionic surfactant, and either
  - (i) adding the nonionic surfactant, if required removing some or all of the organic solvent, and adding water;or
  - (ii) adding a solution of nonionic surfactant in water;the amounts of rapamycin and nonionic surfactant being predetermined to give the desired concentrations in the composition.
2. A process as claimed in Claim 1 wherein the organic solvent is acetone, methanol or ethanol.
3. A process as claimed in Claim 1 or Claim 2 wherein the nonionic surfactant is polyoxyethylated castor oil or polyoxyethylated hydrogenated castor oil.
4. A process as claimed in Claim 1 or Claim 2 wherein the aqueous solution comprises about 1.0 to 20 percent by weight of nonionic surfactant.

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5. A process as claimed in Claim 1 in which the aqueous solution comprises about 1 to 20 percent by weight of a nonionic surfactant selected from polyoxyethylated castor oil and polyoxyethylated hydrogenated castor oil.

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54 **Injectable composition of rapamycin.**

57 Described is an injectable composition comprising rapamycin and a nonionic surfactant selected from polyoxyethylated fatty acids, polyoxyethylated fatty alcohols or polyoxyethylated glycerine hydroxy fatty acid esters and a method of preparing said compositions. The composition provides enhanced blood levels of rapamycin in a mammal following injected administration thereof.

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# EUROPEAN SEARCH REPORT

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)
D, Y	BE-A- 877 700 (AYERST McKENNA et al.) *Claims 1-5*	1	A 61 K 9/00
D, Y	US-A-3 929 992 (S.N. SEHGAL et al.) *Column 4, line 18 - column 5, line 3*	1	
Y	DE-B-1 518 819 (BASF) *Claim; column 5, line 17 - column 6, line 10*	1	
Y	CHEMICAL ABSTRACTS, vol. 71, no. 11, 15th September 1969, page 200, col. 1, no. 47895b, Columbus Ohio (USA); T. MATSUZAWA et al.: "Effect of some nonionic surfactants on the absorption of enduracidin from the muscles". & CHEM. PHARM. BULL. TOKYO 1969, 17(5), 999-1004. *Abstract*	1, 4	
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			A 61 K 9/00 A 61 K 31/00
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 01-06-1982	Examiner WILLEKENS G. E. J.
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

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A	--- "Index of antibiotics from actinomycetes", vol. II, H.Humezawa, Japan scientific societies press, 1978, page 869, Tokyo (JP). -----		
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int. Cl. 3)
Place of search THE HAGUE		Date of completion of the search 01-06-1982	Examiner WILLEKENS G.E.J.
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published or, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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